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Anatomical bases of sex- and size-related acoustic variation in herring gull alarm calls

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The hypothesis that anatomical or physiological factors can constrain the production of vocalizations is supported by an increasing number of examples from a range of taxa, where acoustic variation is related to sex, body-size or condition. In this study, we combine anatomical and acoustic investigations in herring gulls *Larus argentatus* to 1) identify co-variation between sex, body size and the dimensions of the vocal apparatus and 2) test the possible effect of this co-variation on interindividual variation in the acoustics of alarm calls. We found that the vocal apparatus was sexually dimorphic, with males having longer trachea and bigger vibratile membranes than females. We also identified a relationship between the head–bill length – a secondary sexual trait – and the length of the trachea in males only. However, we failed to identify corresponding sex- and body-size related variation in the acoustic components of alarm calls. We suggest that this absence of a relationship between anatomical and acoustic dimensions may reflect the lack of biomechanical constraints exerted during the production of alarm calls, and that such relationships are more likely to be expressed in this species' sexual calls, whose production is characterised by more pronounced, ritualised postures that are more likely to highlight inter-individual size variation.

There is growing evidence that indexical information originating from biomechanical constraints operating on signal production plays an important role in animal vocal communication (Fitch and Hauser 2002, Reby and McComb 2003, Taylor and Reby 2010, Charlton et al. 2011). For example, the acoustic structure of vocalizations conveys information about caller traits such as body size (birds: Appleby and Redpath 1997, anurans: Bee et al. 1999, mammals: Charlton et al. 2009), hormonal status (Beani et al. 1995, Charlton et al. 2012) or age (Ballintijn and ten Cate 1997). In order to evaluate the origins and function of such indexical information, it is crucial to consider how vocal signals are produced.

The source–filter theory, which describes the production of human speech (Fant 1960, Titze 1994), as well as mammal vocalisations (Fitch 2000, Taylor and Reby 2010) can also be extended to the production of bird vocalisations (Fitch and Kelley 2000, Riede et al. 2006). In birds the sound source is the syrinx (equivalent to the larynx in mammals) and the filter is the supra-syringeal vocal tract (composed of the trachea and the oropharyngeal–esophageal cavity, instead of the supra-pharyngeal vocal tract composed of the pharyngeal, oral and nasal cavities in mammals, Fitch and Hauser 2002, Riede et al. 2010). While the morphology of the syrinx varies greatly across species (King

1989), it normally includes vibratile membranes whose rate of vibration determines the periodicity of the sound (hence its fundamental frequency, f_0). The supra-syringeal vocal tract subsequently acts as the acoustic filter that shapes resonance frequencies (tracheal resonances or formants, Fitch and Kelley 2000, Riede et al. 2006) of the radiated signal. Several studies of bird vocal communication have highlighted the importance of source-related parameters (e.g. frequency modulations in collared doves *Streptopelia decaocto*: Slabbekoorn and ten Cate 1997 or fundamental frequency in scops owls *Otus scops*: Hardouin et al. 2007), as well as filter-related parameters (e.g. formants in whooping cranes *Grus americana*: Fitch and Kelley 2000).

Within species, acoustic variation can originate from age, sex or inter-individual variation in the morphology or size of the vocal apparatus. Sex differences in the morphology of the vocal apparatus are common in birds (King 1989). Anatomical structures can be present only in one sex, such as the syringeal bulla in Anatidae (Johnsgard 1971, King 1989) or vary in size between the sexes. In the Strigidae for example, males are smaller than females, but have a larger syrinx (Miller 1934). In contrast, in jungle crows *Corvus macrorhynchos* males have a longer trachea (Tsukahara et al. 2006), and in collared doves (Ballintijn and ten Cate 1997), males have larger lateral tympaniform membranes, despite

the absence of size differences between the sexes in both species. Inter-individual variation in the dimensions of the vocal apparatus has also been documented in several avian species (common eiders *Somateria mollissima* and king eiders *S. spectabilis*: Miller et al. 2007, oilbird *Steatornis caripensis*: Suthers 1994), and is suggested to provide a potential source of acoustic cues to size, condition or identity (Suthers 1994). Sex-, body-size- or condition-related acoustic variation has indeed been reported in several species (blue petrel *Halobaena caerulea*: Genevois and Bretagnolle 1994, Suthers 1994, Hardouin et al. 2007, African black coucal *Centropus grillii*: Geberzahn et al. 2009). However, to our knowledge, very few studies of avian vocal communication have combined investigations at both the anatomical and acoustic level to examine the extent to which sex and inter-individual variation results in corresponding acoustic variation (although see Riede et al. 2010).

The aim of this study was to simultaneously investigate sex and individual differences at anatomical and acoustical levels in herring gulls *Larus argentatus*, a sexually dimorphic species where both sexes produce vocalisations characterised by complex spectral structures including source-related components (fundamental frequency and associated harmonic overtones) and/or filter-related components (formant frequencies). We examined the

relationship between sex, body size variables (wing chord, tarsus, central rectrix, head–bill lengths), body mass and dimensions of the vocal apparatus in adult birds. We predicted a positive relationship between body dimensions and those of the vocal apparatus, both between and within sexes. We then analysed fundamental frequency and resonance frequencies in calls recorded from adult birds of known sex, body size and body mass, in order to investigate how variation in these factors affected acoustic variables. We predicted that both fundamental frequency and formant frequencies would be inversely related to body size (Fitch and Hauser 2002, Hardouin et al. 2007), reflecting sex differences and individual variation in size. We conducted our investigations on two types of alarm calls (kyow-call and kek-calls, Cramps and Simmons 1983), both given from similar upright-postures (Cramps and Simmons 1983), but characterised by very different spectral features (Cramps and Simmons 1983, Teyss  dre 1984, Fig. 1).

Methods

We followed the Association for the Study of Animal Behaviour/Animal Behaviour Society guidelines for the use

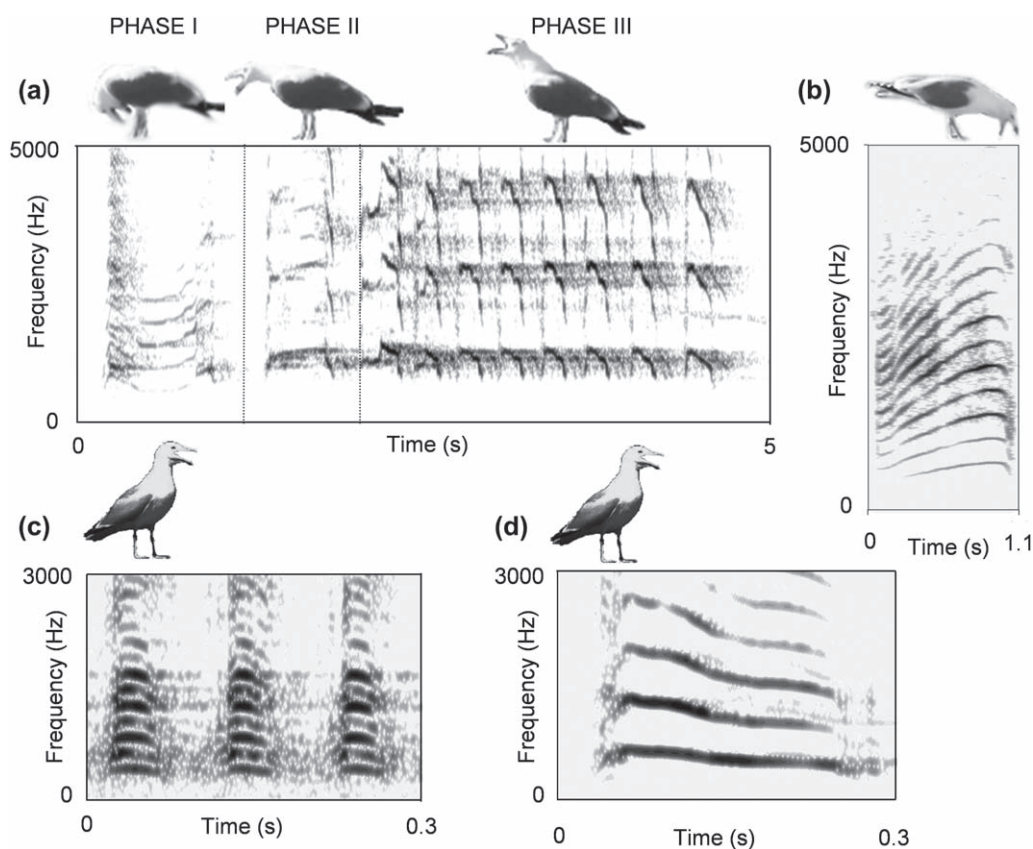


Figure 1. Spectrograms showing the spectral structure of the four most common calls of the adult herring gull repertoire with the different postures adopted during calling. (a) The long call or 'trumpeting call' is composed of three distinct phases, corresponding to particular postures: I) a couple of low hoarse units given with the head pointed down and stretched backward, II) up to three high units given with the head being pointed down and, III) up to 25 identical loud units given with the head stretched far forward and a widely opened beak. (b) The mew call is close to the phase I of the long call and corresponds to a low hoarse unit given with the head pointed down. The kek alarm call (c) and the kyow alarm calls (d) are both given in the upright posture (see Fig. 3 for the spectral description).

of animals in research. The project was conducted under British Trust for Ornithology licence number 5396 to LAH.

Relationships between sex, body size and dimensions of the vocal apparatus in specimens

Specimen collection

The 41 herring gull specimens that we studied were provided by the Royal Society for the Prevention of Cruelty to Animals, Mallydams Wildlife Centre (Fairlight, Hastings, county of East Sussex, UK). About 500 injured herring gulls are rescued and treated each year (mainly during the breeding season) by the Centre and individuals with fatal or incurable injuries are euthanized with sodium pentobarbitone Ph.Eur. 200 mg ml⁻¹ Pentoject, via intravenous (IV; 0.4 ml kg⁻¹) or intraperitoneal (IP; 0.8 ml kg⁻¹) injection. All specimens were euthanized and frozen in 2006 and 2007. We excluded diseased animals (with clearly identifiable pathologies or lacking obvious wounds) in order to avoid biasing our sample with a disproportionate number of specimens in poor condition. Because all the specimens were subjected to the same freezing and thawing procedures, so we assumed that any alterations to elasticity or dimensions of the tissues were consistent across samples (Miller et al. 2007, 2008). After measuring the specimens we removed the head, trachea and bronchia of dead gulls from the rest of the body and froze them in freezer bags (storage duration inferior to 9 months at -18°C). All specimens were destroyed after syringeal and tracheal measurements were taken.

Measurement of body dimensions

We measured five variables characterising body size (Table 1): body mass (± 1 g), and the lengths of the wing chord, central rectrix, right tarsus (precision ± 1 mm) and head–bill (measured from the tip of the bill to the posterior margin of occipital protuberance; precision ± 0.1 mm). We then used principal component analysis to derive an index of body size based on wing chord, rectrix and tarsus lengths. While average body size in male herring gulls is larger than in females, there is a substantial overlap in body size between the sexes (Coulson et al. 1983, Burger 1984). However males tend to have squarer, longer and larger heads (Tinbergen 1953), and the head–bill length can be used as a reliable predictor of sex (Coulson et al. 1983). Measures of head–bill lengths in our sample of euthanized specimens (sexed by visual examination of the reproductive organs during dissection) confirmed the reliability of this measure as a predictor of sex in our population (all the males had head–bill length over 119 mm, and all females had head–bill lengths below 119 mm). We therefore used this measure to sex live animals when captured. We estimated age using plumage and bill states (following Olsen and Larsson 2004). All selected males and females were sexually mature in their summer morphs (i.e. white head, bill bright yellow with red gonys-spot and fleshy to reddish orbital ring), and were at least four years old.

Measurement of the dimensions of the vocal apparatus

King (1989) provided a detailed description of the herring gull's tracheobronchial syrinx (see also Rüppell 1933, Fig. 2):

the syrinx is composed of five to six tracheosyringeal cartilages that are merged to form the tympanum. The lateral tympaniform membranes (LTM) are located between the first (bs1) and second bronchosyringeal cartilages (bs2). In dorsal view, the medial tympaniform membranes (MTM) span the six bronchosyringeal cartilages (that consisted of C-shaped semi rings, visible in ventral view, that are interrupted in dorsal view, leaving a large space from which the MTM stretched).

We pinned the frozen head to a board. We moistened the specimens with a water mist-spray and left for 5 min, and then subsequently every 5 min. After a qualitative assessment of the states and quality of the thawed tissues, we discarded damaged specimens. We measured 20 males and 10 females. To standardise the measurement of tracheal length, we measured the compressed and extended tracheal lengths (Miller et al. 2007). We measured the compressed tracheal length after fully compressing the trachea longitudinally by hand and measured the extended tracheal length, after 1) hooking the bronchi to a 60 g spring scale (precision $\pm 0.3\%$), 2) stretching the trachea with a tension of 20 g (Miller et al. 2007) and 3) pinning the spring scale to the board. Similarly, to standardise the tympaniform membrane measurements and lateral dimensions of the trachea, we hooked each bronchus (at the 4th and 5th bronchus ring) to a 60 g spring scale, exerted a tension of 20 g on each bronchus and pinned the spring scales to the board (we spread apart the spring scales at a 45° angle). We chose this tension as optimal for reproducing the natural position of the syrinx and trachea observed *in vivo* before the dissection. Vocal apparatus measurements were performed with a calliper (precision ± 0.02 mm), except for trachea length, which were performed with a ruler (precision ± 1 mm). Tracheal measurements included the cranio–caudal lengths of the compressed/extended trachea from the first (after the larynx) to the last (before the tympanum) tracheal rings as well as the width of first and last tracheal rings. Left and right syringeal measurements included (Fig. 2): 1) width of the bronchus at the level of bs1 (hereafter LTM width), 2) maximum distance between bs1 and bs2 (hereafter LTM length), 3) width and 4) length of MTM (from the measurements 3 and 4 we estimated the surface of these well-defined elliptic shaped membranes).

We measured tracheal and syringeal variables twice on a randomly selected sample of 10 individuals. The second measurements were taken an hour after the first (specimens were kept moistened in the meantime). The order of the specimens was randomly chosen and the measurer was blind to the specimen identity. We used a nested ANOVA with repeated measures (1 and 2) nested within specimen ID to assess for the repeatability (r). We used the following formula $S^2A/S^2 + S^2A$ where S^2A is the between group variance and S^2 is the within group variance. Repeatability was high ($r > 0.99$ for all measurements).

Relationship between body dimensions and acoustic variables in live animals

Study population

We studied the vocal behaviour of free-ranging gulls on the campus of the Univ. of Sussex, where about 30 pairs bred on the flat roofs of the main buildings in 2007. We captured

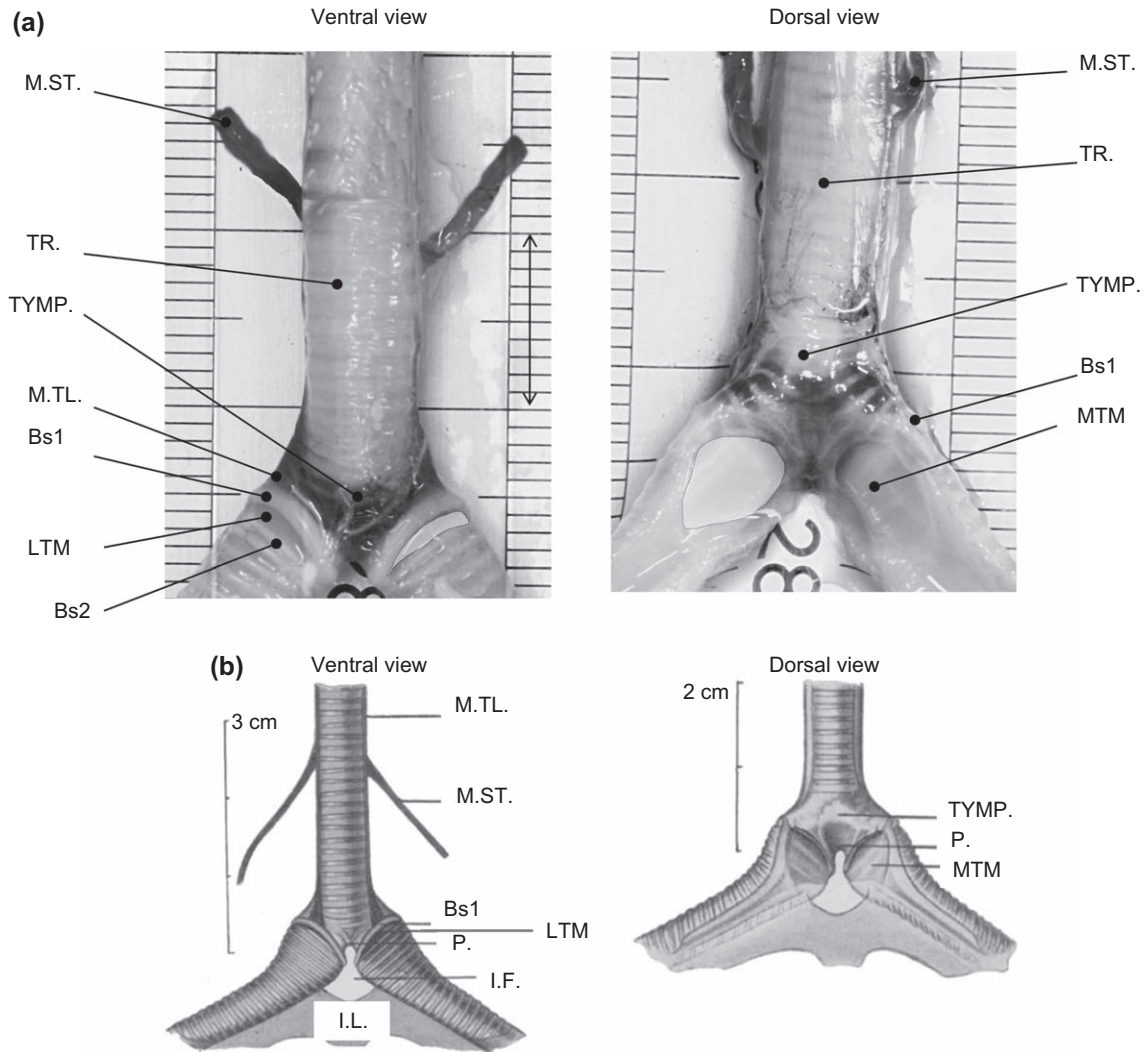


Figure 2. (a) Photography of the syrinx of male herring gull (*Laridae*, *Charadriiformes*) and (b) drawing of the syrinx of female herring gull adapted from Rüppell (1933). The ventral (left side) and dorsal view (right side) are represented. M.ST.: sternotrachealis muscle, M.TL.:tracheolateralis muscle, TR.: trachea, TYMP.: tympanum, LTM: lateral tympaniform membrane, Bs1: first bronchosyringeal cartilage, Bs2: second bronchosyringeal cartilage, MTM: median tympaniform membrane, P.: Pessulus, I.F.: interbronchial foramen, I.L.: interbronchial ligament. The right MTM and LTM shapes are drawn on the photography (white shapes) and the double arrow indicates 1 cm.

(using whoosh net or walk-in cages) and ringed (using both colour alphanumeric Darvic and metal BTO rings) a total of 32 adults. Animals were aged and measured and released within 15 min of capture. All body measurements were the same as for the euthanized specimens. All animals were in their summer morphs at the time of capture and at least 4 yr old.

Description of call types and recordings

The vocal behaviour of herring gulls is relatively similar between sexes (Tinbergen 1953, Burger 1984, Teyss  dre 1984). Four calls are particularly common: the long and mew calls that are produced in mating, bonding and/or territorial defence contexts, and the kek- and kyow calls that are produced in contexts of danger or vigilance (Fig. 1). Most calls are spectrally complex, with clear harmonic structure and resonance frequencies in the lower-pitched vocalisations

(Fig. 1). During long-calling and mew-calling, males and females adopt stereotyped display postures (e.g. during the long call the head and the neck are fully stretched forward, the mew call is produced with the head and neck pointed down, Fig. 1). Such postural behaviour may affect the source- and filter-related variables by respectively modifying the tension of the tympaniform membrane, or the tracheal length (Riede et al. 2006). Alarm calls (Fig. 1, 3) are produced while in an upright-posture (i.e. vertically extended neck and horizontal bill, in apparent continuum from relaxed postures). The kek-call has low fundamental frequency with a well-defined harmonic structure and clear tracheal resonances. In contrast, the kyow call is characterised by a high fundamental frequency resulting in low spectral density and poorly resolved tracheal resonances. We recorded kek and kyow alarm calls, both elicited by the presence of a human observer, from sixteen adult ringed gulls using a shotgun microphone

and a solid-state recorder on compact flash cards (file format: WAV, 22 kHz, 16 bits). We selected between one and nine calls from 25 series of kyow calls recorded from six males and four females (total: 134 kyow calls). We also selected between two and 66 syllables from 40 series of kek calls recorded from eight males and eight females and (total: 723 syllables from 197 kek calls).

Acoustic analyses

Recordings were edited and high-pass filtered (Stop Hann Band: min = 0 Hz, max = 500 Hz for kyow calls and 100 Hz for kek calls) with PRAAT 5.3.0.4 (Paul Boersma and David Weenink, Univ. of Amsterdam, the Netherlands). The frequency contour of kyow (Fig. 3d) and kek calls (Fig. 3c) was extracted using the 'to pitch cc' command in PRAAT with the following settings: time step, 0.001 [s], silence threshold: 0.03, minimum F = 800/200 Hz, maximum F = 2000/500 Hz (accuracy of frequency discrimination: ± 0.01 Hz). The following variables were used to characterise the frequency contour of both calls (Fig. 3c–d): maximum frequency of the contour $maxf_0$ (at onset of

call), the minimum frequency of the contour $minf_0$ (at end of call) and mean frequency $meanf_0$. While the kyow alarm call has a relatively high fundamental frequency (Fig. 3b) without clear vocal tract resonances, the kek alarm call has a relatively low fundamental frequency and well-defined resonances (Fig. 3a). Formant frequencies were therefore only measured in kek calls. We measured the centre frequencies of the first four formants (f_1 , f_2 , f_3 , f_4 ; Fig. 3a) and used these to estimate Δf (Table 4). The centre frequency values of the first four formants were extracted using linear predictive coding (LPC) via the 'LPC: to formant (Burg)' command in PRAAT (Fig. 3a). Our analysis parameters were: time step, 0.001 s; maximum number of formants, 6; maximum formant frequency, 8000 Hz; window of analysis, 0.025. We assessed and determined these settings following initial spectrogram inspections. We extracted the frequency and formant parameters semi-automatically (following visual inspection, we adjusted formant analysis parameters and corrected spurious pitch in the presence of octave jumps). We then derived the overall formant frequency spacing, Δf , from the frequencies

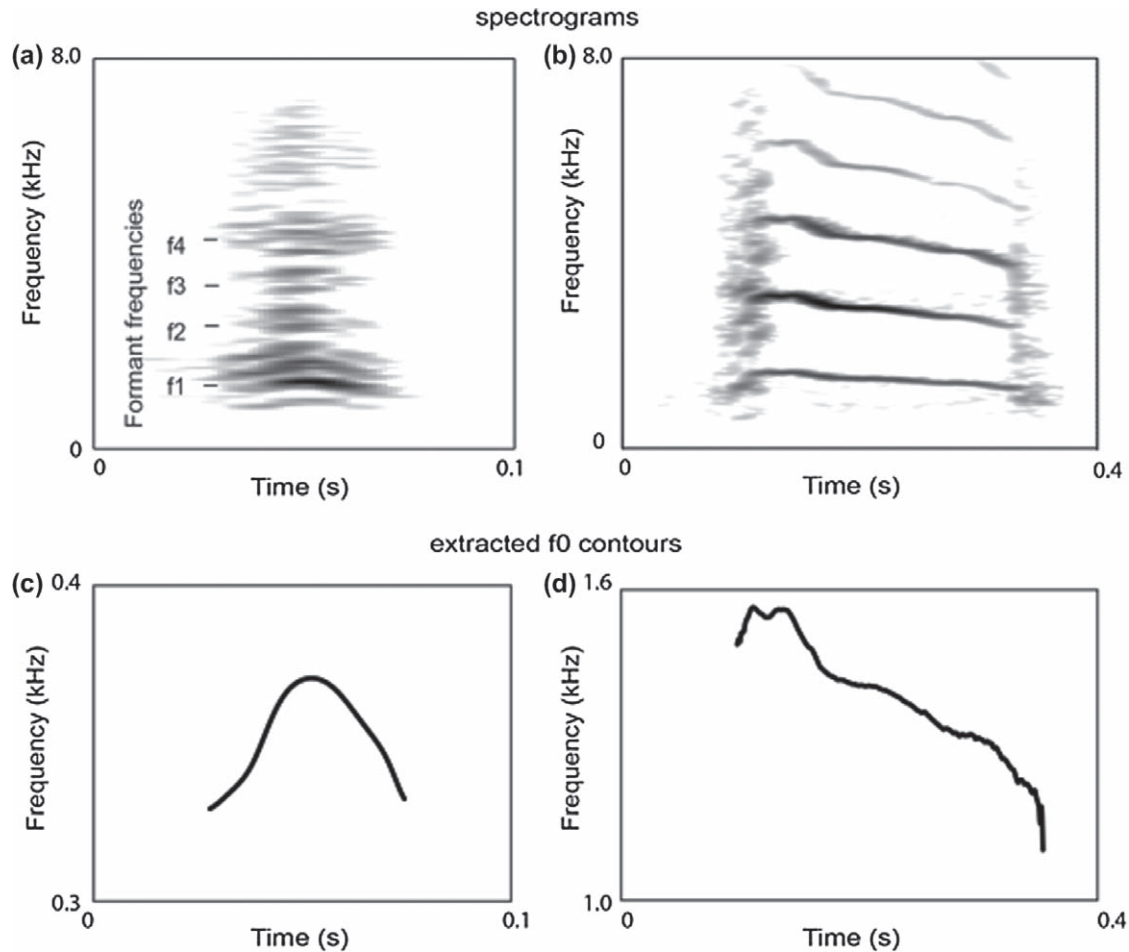


Figure 3. (a): narrow band spectrogram of a kek call given by a female herring gull. Kek calls have a relatively low f_0 and well defined formant frequencies (labelled f_1 to f_4 on the spectrogram). (b): narrow band spectrogram of a kyow call given by a herring gull male. Kyow calls are characterised by a much higher fundamental frequency and poorly defined formants (due to the low spectral density associated with the high f_0). (c): corresponding fundamental frequency contour for the kek call and (d): corresponding fundamental frequency contour for the kyow call. These numerical representations extracted using the 'to pitch' command in PRAAT were used to calculate f_0 -related variables.

of the first four formants by finding the best fit for the following equations:

$$F_i = \frac{2i-1}{2} \Delta F \quad (1)$$

which relates individual formant frequencies (F_i where 'i' refers to the formant number) to average overall formant spacing (Δf) in a vocal tract approximated as a straight uniform tube closed at one end (the syrinx) and open at the other end (i.e. the bill); and

$$F_i = i \times \Delta F \quad (2)$$

which relates individual formant frequencies to overall formant spacing in a vocal tract approximated as a straight uniform tube open at both ends. In both cases the intercept was set to 0 (see Reby and McComb 2003 for details and justification of this procedure).

We estimated R^2 values to compare the fit of the open/closed and open/open models to the observed distribution of the vocal tract resonances. We chose the open/open model because R^2 was significantly higher ($F_{1,1492} = 1033$, $p < 0.001$) than in the open/closed model. In the final step, we deduced the estimated apparent vocal tract length (VTL) directly from formant spacing Δf from the following equation:

$$VTL = \frac{c}{2\Delta F} \quad (3)$$

where c (350 m s^{-1}) is an estimate of the speed of sound in a vertebrate vocal tract.

Statistical analyses

We evaluated the bilateral asymmetry of the syrinx using a linear mixed model testing for the interaction effect of the side (left vs right) of the syrinx and sex on morphometric variables, with individual as random factor. We then assessed relative sex differences in the dimensions of the vocal apparatus using linear models for tracheal dimensions (with morphometric variables as outcome variables, sex as a fixed factor and our index of body size (PC1) as a covariate, and a linear mixed models for syringeal dimensions (with each morphometric variables as outcome variable, specimen and side of measure as random effects, sex as a fixed factor and PC1 as a covariate). We also examined relationships between body size index, head–bill length, body condition

and morphometric of the vocal apparatus within males and females using simple regressions. As 39 correlations were tested for each sex, we only report the more relevant results. Because these correlations tested specific hypotheses, we did not apply Bonferroni corrections to adjust the significance threshold (Perneger 1998).

To assess the relationship between body-size index/head–bill length/body condition and acoustic variables, we ran mixed-effect models using acoustic characteristics ($meanf0$, $minf0$, $maxf0$ and Δf of the kek call) as outcome variables and body condition, body size index, head–bill length and first-order interaction with sex as predictor variables. To account for repeated measures, we nested individual identity in the recording session for the kyow call and nested individual identity in the recording session and the call identity (as we took several syllables within the same call) for the kek call. We added year of recording as a crossed random factor. Because appropriate degrees of freedom in mixed-effects models are controversial (Baayen 2008), we used Markov chain Monte Carlo (MCMC) sampling with 100 000 samples and present model coefficients with estimates of highest posterior density (HPD) intervals. With this procedure, a coefficient is deemed significantly different from zero when the HPD interval does not include zero. After MCMC sampling, we simplified the random factor structures of the models to include only individual identity because recording session, year and call identity HPD estimates were all non-significant. We performed statistical analyses using R ver. 2.10.1 with libraries lme4 (Bates and Maechler 2009) and languageR (Baayen 2009).

Results

Sex differences in body size

Males were significantly larger (wing, tarsus and rectrix length), heavier (body mass) and had longer head–bill length than females, both in dead specimens and live birds (Table 1). Because wing, tarsus and rectrix length were strongly correlated (Table 2), we performed a principal component analysis (PCA) in order to calculate an index of body size (after pooling the measurements from dead specimens and live birds). The first principal component (PC1) had an eigenvalue greater than 1 and was strongly correlated ($r < -0.83$) with all three body-size variables (Table 2). An index of body condition was then calculated as the residual of the regression of body mass on PC1 and

Table 1. Body measurements of adult male and female herring gulls, recorded from dead specimens and captured free-ranging birds. The results of linear generalized model are given with body measurements as response variables, individual types (specimens or free-ranging) and sex as fixed factors. All measurements are given in mm, and body mass in grams.

n	Specimens		Free-ranging		Anova of the model			Specimens vs Free-ranging		Male vs female	
	Male 27	Female 14	Male 14	Female 16	R^2 adj.	$F_{2,68}$	p	t-value	p	t-value (sex)	p (sex)
Wing length	413 ± 20	397 ± 15	415 ± 13	402 ± 12	0,13	16,1	0.002	0,9	0,4	3,6	<0.001
Tarsus length	76.4 ± 2.2	70.8 ± 2.2	76.2 ± 2.9	71.4 ± 2.8	0,5	36,4	<0.001	0,2	0,8	8,4	<0.001
Rectrix length	168.5 ± 4.9	157.3 ± 7.1	167.1 ± 7.2	158.9 ± 6.4	0,37	21,9	<0.001	−0,04	0,9	6,5	<0.001
Head plus bill length	122.5 ± 2.9	112.1 ± 2.4	122.0 ± 3.5	112.7 ± 3.4	0,72	90,6	<0.001	−0,04	0,9	13,2	<0.001
Body mass	902 ± 73	759 ± 65	900 ± 60	744 ± 52	0,57	47,7	<0.001	−0,4	0,6	9,5	<0.001

Table 2. Principal components analysis performed on the three body size variables (top section), correlation matrix of body-size related variables (lower left section) and correlation coefficients of the body-size related variables with the eigenvalues of the three components (lower right section).

	Body-size measurements			Components		
	Wing chord	Tarsus length	Central rectrix	1	2	3
Eigenvalues	–	–	–	1.49	0.67	0.56
Percentage of variance	–	–	–	74.2	15.1	10.7
Cumulative percentage	–	–	–	74.2	89.3	100
Body-size measurements						
Wing chord	1	0.55	0.67	–0.86	0.37	–0.33
Tarsus length	–	1	0.61	–0.83	–0.54	–0.11
Central rectrix	–	–	1	–0.88	–0.11	–0.43

sex ($F_{2,68} = 70.3$, adj. $R^2 = 0.66$, $p < 0.001$). This index of body condition did not differ between our samples of euthanized specimens and free-ranging birds (t -value = -0.9 , $p = 0.4$). Finally after correcting for overall differences in body size between the sexes (by using PC1 as covariate in the model), head–bill length was disproportionately longer in males than females ($F_{1,68} = 119.7$, $p < 0.001$).

Morphometrics of the vocal apparatus and correlations with sex and body size

There were no significant asymmetries in the syrinx of either sexes ($p > 0.07$ for side and $p > 0.4$ for side \times sex interaction in all tested variables). We present the mean \pm SD of the different measurements of the trachea and tympaniform membranes in Table 3. Direct comparisons (without controlling for sex difference in body size) between sexes indicated that all measurements were significantly greater in males than females (Table 3).

Once these comparisons were controlled for body size, we found that the MTM vibratile membrane was disproportionately broader in males than in females (Table 3). The extensibility of the trachea (measured as the ratio of the fully

extended trachea and compressed) was 51–68% (median = 57%) in males and 50–59% (median = 55%) in females and was comparable to that reported by Miller et al. (2008) in murres (57–78%, median = 67%). Both the compressed and extended tracheas were disproportionately longer in males than the female (Table 3, 11.3% and 6.5% respectively). While the trachea was wider in males than females at the cranial end, the width at the caudal end was similar between sexes (Table 3).

The effect of the interaction between sex and head–bill length on the compressed trachea is significant (t -value = 2.42, $p = 0.02$), meaning that the relationship between head–bill length and compressed trachea length differed between males and females. More specifically, the compressed trachea length was positively correlated with the head–bill length in males only ($F_{1,19} = 12.8$, R^2 adj. = 0.37, $p = 0.001$). However, all other correlations between body size, body condition, head–bill length and dimensions of the vocal apparatus in males and females were non-significant (all $p > 0.06$).

Sex differences and size-related variation in the acoustic features of the kyow- and kek-calls

There were no significant sex differences in any of the investigated acoustic features (Table 4). The sex difference in Δf estimated from formant frequencies measured in kek-calls was small (observed sex difference in Δf of 26 ± 21 Hz) and non-significant. This lack of sex difference in Δf is consistent with the absence of sex difference predicted by applying Eq. 3 to the extended tracheal length (predicted sex difference in Δf : 58 ± 16 Hz, Δf observed vs Δf predicted: t -value = -0.9 , $p = 0.4$). This however contrasts with the significant sex differences predicted by the sex difference in length observed for the compressed tracheal length (predicted sex difference: 182 ± 29 Hz, Δf observed vs Δf predicted: t -value = -4.1 , $p < 0.001$). Finally, we found no significant relationship, neither in males nor in females, between call acoustic variables (meanf0, maxf0, minf0 and Δf) and body-size (PC1), head–bill length or body condition (all HPD95 included 0 with all $p > 0.07$).

Table 3. Tracheal and syringeal measurements (width and lengths in mm, given as mean \pm SD) in 20 adult male and 10 adult female herring gulls. Results of linear (for the trachea) and mixed (for the syrinx) models with vocal apparatus measurements as response variable, sex and body size (i.e. PC1) as fixed factors. LTM = lateral tympaniform membranes, MTM = medial tympaniform membranes.

	Male and female differences					
	Dimensions of the vocal apparatus		Not controlled for body size		Controlled for body size	
	Male	Female	t-value	p	t-value	p
Compressed trachea length	122 \pm 7	108 \pm 5	5.5	< 0.001	2.9	0.006
Extended trachea length	210 \pm 11	196 \pm 9	3.5	0.001	2.2	0.03
Trachea width-cranial end	10.78 \pm 0.52	8.96 \pm 0.42	9.9	< 0.001	5.8	< 0.001
Trachea width-caudal end	6.29 \pm 0.33	5.73 \pm 0.46	3.9	< 0.001	1.6	0.1
Width of LTM	8.70 \pm 0.43	8.30 \pm 0.32	2.7	0.009	0.65	0.5
Length of LTM	1.63 \pm 0.16	1.46 \pm 0.16	3.6	< 0.001	1.8	0.08
Width of MTM	7.49 \pm 0.63	6.71 \pm 0.53	3.7	< 0.001	2.6	0.01
Length of MTM	7.25 \pm 0.47	6.68 \pm 0.34	3.7	< 0.001	1.8	0.07
Estimated area of MTM (mm ²)	42.7 \pm 4.6	35.2 \pm 3.3	4.9	< 0.001	2.9	0.004

Table 4. Sex differences in the acoustic variables of the kyow and kek calls. Values are reported as mean \pm SD. ANOVA F and p-values are reported.

Sample	Kyow calls				Kek call			
	Female 4	Male 6	Anova		Female 8	Male 8	Anova	
			F _{1,8}	p			F _{1,14}	p
Fundamental frequency								
Mean F0 (Hz)	1379 \pm 107	1404 \pm 60	0.2	0.6	367 \pm 28	358 \pm 20	0.5	0.5
Maximum F0 (Hz)	1512 \pm 92	1554 \pm 79	0.6	0.4	383 \pm 31	378 \pm 23	0.2	0.7
Minimum F0 (Hz)	1199 \pm 137	1202 \pm 36	0.002	0.9	339 \pm 19	327 \pm 16	1.8	0.2
Formant frequencies								
F1	–	–	–	–	1261 \pm 100	1219 \pm 43	1.2	0.3
F2	–	–	–	–	2010 \pm 131	1958 \pm 138	0.6	0.4
F3	–	–	–	–	3206 \pm 124	3233 \pm 140	0.1	0.7
F4	–	–	–	–	4510 \pm 224	4337 \pm 202	2.6	0.1
Delta F	–	–	–	–	1098 \pm 37	1072 \pm 45	1.4	0.2
Estimated tracheal length	–	–	–	–	15.9 \pm 0.5	16.4 \pm 0.7	1.5	0.2

Discussion

Our anatomical investigations revealed a number of significant between-sex differences in the dimensions of the vocal apparatus, as well as within-sex correlations between body size and dimensions of the vocal apparatus, leading us to make specific predictions regarding the co-variation of acoustic variables with sex and body size. However, none of these predictions were met, suggesting that, at least for the two alarm calls considered in this study, there is no effect of sex and individual variation in the dimensions of the vocal apparatus on the acoustic components of the calls, or that any such effect is too small to be revealed by our relatively small sample size (especially for females).

At the level of the syrinx, we found that the medial and lateral tympaniform membranes were broader in males than in females. This observation led us to predict that, assuming the tympaniform membranes are involved in sound production in Laridae, males may produce calls with a lower f0 than females. However, we failed to identify such a sex difference in the f0 of alarm calls. This suggests that differences in dimensions of the tympaniform membranes of the magnitude of those observed between the sexes do not affect the fundamental frequency of alarm calls in herring gulls. While similar sex dimorphism in the dimensions of the tympaniform membranes have been reported in other sexually dimorphic non-passerine species including owls, grouse and eiders (Miller 1934, Degner 1988, Miller et al. 2007), their consequences on the vocal signals of these species has not yet been investigated. Within sexes, the size of the tympaniform membranes was not correlated with body size, head–bill length or body condition. The fact that the fundamental frequency did not co-vary with any of those size-related variables, in either type of alarm calls, is consistent with these observations. While negative relationships between body size and fundamental frequency have been reported in other species (scops owls: Hardouin et al. 2007, African black coucal: Geberzahn et al. 2009), these studies focused on sexual calls rather than alarm calls.

At the level of the vocal tract filter, while head–bill and excised trachea lengths (both compressed and extended) were all relatively longer in males than in females, these

anatomical sex differences did not translate into significant differences in vocal tract resonances measured in the kek alarm call. Similarly, while the compressed trachea length was positively correlated with the head–bill length in males, this relationship did not affect inter-individual variation in resonance frequencies, suggesting that the tracheal measures in dead specimens may not constitute suitable predictors of acoustic variation. We did not investigate the potential for dynamic adjustments of the volume of the oropharyngeal-esophageal cavity, which is known to play a role in vocal tract filtering in several species (e.g. ring doves: Riede et al. 2004, northern cardinals: Riede et al. 2006, white-throated sparrows *Zonotrichia albicollis*: Riede and Suthers 2009). However, the apparent length of the trachea achieved during the production of kek calls (i.e. the vocal tract length estimated from the vocal tract resonances frequencies in kek calls) falls between the compressed and extended trachea measurements, suggesting that dynamic adjustments of the oropharyngeal-esophageal cavity are not necessary to interpret the vocal tract resonances observed in this alarm call. However, the role of oropharyngeal-esophageal cavity should be investigated in herring gull sexual calls, as their production is associated with an inflation of the neck area (unpubl.). The relatively intermediate upright postures adopted by herring gulls during the production of both alarm calls contrast with the more dynamic and extreme postures adopted during long and mew-calling (Fig. 1). It is therefore possible that, when vocalising in the upright position, the dimensions of the trachea are poorly constrained by overall animal body size, therefore limiting the potential for tracheal resonances to automatically encode body size variation. We suggest that the more pronounced postures achieved during sexual calling displays (head fully pointed down and then fully stretched backward, or head stretched forward) may reveal variation in body size, and may amplify sex differences in the resonance frequencies of the sexual calls (i.e. mew and long calls, Fig. 1). Future studies should also investigate the extent to which postural variation dynamically influences the acoustic features during the production of herring gull sexual calls, as well as the co-variation these features with sex and inter-individual variation in body size.

While relationships between body size and resonance frequencies are common and well documented in mammals (Riede and Fitch 1999, Reby and McComb 2003, Vannoni and McEligott 2007, Charlton et al. 2009, 2011), very few studies have identified equivalent relationships in birds (zebra finch *Taeniopygia guttata*: Riede et al. 2010). Experimental evidence indicates that whooping cranes *Grus americana* can perceive size-related variation in tracheal resonances (Fitch and Kelley 2000), and that ravens *Corvus corax* lower their tracheal resonances in response to non-affiliates (Boeckle and Bugnyar 2012). Furthermore, the evolution of tracheal elongation in several species of birds has been suggested to result from selective pressures favouring size exaggeration in these species (Fitch 1999). While these observations converge to suggest that vocal tract resonances function as cues to body size in birds, further research is clearly needed to establish the extent to which this assumption is verified in calls from a wider range of avian species.

In conclusion, while we identified size-related differences in some dimensions of the vocal apparatus of euthanized herring gulls, we failed to identify corresponding differences in the acoustic structure of the two alarm call types in living animals. We suggest that this result may reflect the absence of selective pressures to emphasise sex and size differences in the alarm calls of this species. Instead, strong inter-individual variation in source and filter-related components may encode information on arousal and urgency, as reported in the alarm call of other vertebrate species (Manser 2001). Future investigations should therefore investigate whether this absence of correlation also extends to sexual calls in this relatively monomorphic and monogamous species, with reciprocal vocal displays shared by both sexes.

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